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Cell Proliferation and Carcinogenesis: A Brief History and Current View Based on an IARC Workshop Report

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The International Agency for Research on Cancer recently convened a Working Group of Experts (June 11–18, 1991) to discuss the use of information on carcinogenesis mechanisms in carcinogenic risk identification. The role of cell proliferation in carcinogenesis was among the items discussed in detail. It was recognized that cell proliferation is an important mechanistic aspect for both genotoxic and nongenotoxic carcinogens. It may act at each stage of the carcinogenesis process, altering the size of the pool of cells at risk for a next event. Cell proliferation was considered to be important, especially as *a*) an integral part of the process of converting DNA adducts to mutation, *b*) an enhancing factor for the mutation frequency by inducing errors in replication, and *c*) an important factor in determining dose–response relationships for some carcinogens. It was also recognized that not all agents that induce cell proliferation are necessarily involved in carcinogenesis; for example, *a*) not all skin hyperplasia-inducing compounds are skin tumor promoters, *b*) agents that induce "regenerative" cell proliferation appear to have different effects on tumor induction from agents that have a direct mitogenic effect, and *c*) the carcinogenic activity of many nonmutagenic agents depends on the continuous administration of the agent. In addition, tissues with a high rate of cell proliferation do not have a higher risk of developing cancer. Thus, no simple relationship exists between cell proliferation and carcinogenesis.

The International Agency for Research on Cancer (IARC) convened an ad hoc Working Group of scientists in Lyon in June 1991, 8 years after the first meeting on the same subject (*1*), to consider and advise on the possible use of mechanistic information in the evaluation of carcinogenicity (*2,3*). One of the outcomes of the meeting, the proceedings of which are now available as an IARC Technical Report (*2*), has been the updating of the Preamble to the IARC Monographs, with some guidelines and indications on how to use mechanistic information in the final qualitative evaluation of carcinogenicity. Information on mechanisms may play an essential role when there is a need to extrapolate from experimental data to the human situation in the absence of epidemiological data. One could assume that humans could be the most affected or susceptible species or the putatively less affected or unaffected species.

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Does cell proliferation modify the carcinogenesis process? The obvious answer is that there would not be a carcinogenesis process without cell proliferation and that cell proliferation is an essential component of the process. Two other questions would then need to be answered: Is cell proliferation carcinogenic per se? Should investigations on cell proliferation be included in routine tests on chemicals?

The dominant hypothesis today is that neoplastic development is a multistage progressive process involving multiple genetic changes. Events and changes that in the past were predicted in experimental and epidemiological studies and remained largely theoretical for decades can now be investigated and challenged in molecular studies. There has therefore been a definite, important advancement in our understanding of carcinogenesis, in spite of some uncertainties.

Some of the initial changes and events of the carcinogenesis process may occur prenatally and/or may involve the germ cells. The extent of the contribution of preconceptional events to what is seen in later generations as predisposition to cancer, or to certain cancers, needs to be better defined. The fact that one or more steps of the carcinogenesis process may occur as

distant in time as in previous generations adds further difficulty to correctly assessing risk (*4,5*).

The possible role of hyperplasia in carcinogenesis has been discussed for almost a century and a half. According to the old theory of irritation as the cause of cancer, neoplasia was a kind of extension of hyperplasia. As a natural follow-up of his fundamental discovery of cellular pathology, Virchow also held the view, against the then-prevailing opinion that tumors were related to discrasia, that tumors mainly originated following the action of local causes (*6*). Virchow supported the essential role of gross mechanical insults in the origin of tumors until the end of his life (*6*). Similar, although not identical, to the irritation theory was the traumatic theory of the origin of tumors which Cohnheim, the most famous pupil of Virchow, was ready to dismiss (*7*). Cohnheim supported the hypothesis that tumors originated from undifferentiated embryonal cells persisting in many adult tissues. The embryonic character of these cells endowed them with a "marked capacity for proliferation" (*7*). This is perhaps the first strong statement about the role of cellular proliferation in tumor development.

The irritation theory was abandoned when studies on tumor formation went beyond the interpretations based on morphological observations alone. Then came a long series of studies on inhibition and augmentation of carcinogenesis. It was shown that agents that stimulate or favor cell proliferation were efficient in enhancing carcinogenesis when acting after initiation. These were studies of Friedewald and Rous (*8*), Mottram (*9*), Berenblum (*10,11*), Berenblum and Shubik (*12*), Boutwell (*13*), and Foulds [(*14*); see also Slaga et al. (*15*)]. Tannenbaum showed that caloric restriction had an inhibiting effect on carcinogenesis acting on the promotion, but not on the initiation of skin carcinogenesis (*16*). The conclusion of all these studies seemed clear: a stimulation or inhibition of cell proliferation had an effect only on late stage(s) of carcinogenesis, but not on the early events.

It is generally accepted that increased cell proliferation, in order to be most effective in enhancing carcinogenesis, must act either on an already modified cell population, or in concurrence with an agent causing specific changes in the cells. Typically, this may occur when a genotoxic agent is administered at the dose that also induces considerable cell proliferation in the target organ. There is indeed definite evidence that many carcinogens damage cellular DNA and cause cell proliferation, exhibiting a combined genotoxic and mitogenic action (*17*). It is not evident that increased cell proliferation plays a role in enhancing carcinogenesis when it occurs before DNA damage; however, because of the small risk of spontaneous mutation (an error in DNA replication occurring for unknown reasons), there will never be a zero probability of initiating the process of carcinogenesis.

Cell proliferation can be induced following cell necrosis and is then called "regenerative" or "compensatory" cell proliferation. Cell proliferation may also be induced directly by some chemicals without causing any cell death and is called "mitogen-induced" cell proliferation. It has been claimed, however, that regenerative cell proliferation subsequent to cell necrosis can be carcinogenic per se, implying that it could have such an effect on a cell population in which an initiating event has not occurred. One of the most quoted examples is that of kidney tumors as a consequence of the α 2μ-globulin nephropathy induced in male rats by a variety of chemicals (*18*). The nephropathy is characterized by tubular cell necrosis and regenerative cell proliferation in the P_2 section of the tubules. However, the chemical 1-(aminomethyl) cycloexane acetic acid (gambetin), which causes typical α 2μ-globulin nephropathy in male rats, has been reported not to produce renal cancer after 2 years of exposure (*19*). Cell necrosis and regeneration induced by nephropathy may therefore be a contributing factor but not a sufficient cause of neoplasia.

A further indication of the complexity of the relationships between toxic injury and neoplasia comes from the review of carcinogenicity results obtained with chemicals tested by the National Toxicology Program (*20–22*). Based on morphological observations, mutagenic as well as nonmutagenic chemicals were shown to cause toxic injury in a variety of tissues without associated neoplastic effects at the same sites or to cause tumors at sites where no associated toxic injury was observed.

Chemicals may indeed exert different types of toxic injury that may not necessarily be related. For instance, asbestos induces asbestosis, which is unrelated to mesothelioma, vinyl chloride induces acro-osteolysis, which is unrelated to liver tumors, and a number of chemicals that cause teratogenicity and carcinogenicity when given prenatally produce teratogenic and carcinogenic effects only occasionally at the same target organ(s).

In several instances it was shown that the carcinogenic activity of agents labeled as mitogens depends on continued administration of the agent and, furthermore, that preneoplastic foci regress when the chemical exposure is interrupted. A particular case of mitogenic action is that of hormones, which is related to receptor-mediated events. The interpretation of this particular mitogenic action is rendered somewhat more complicated by the fact that certain environmental and synthetic chemicals bind to the estrogen and other receptors and elicit hormonal response. Furthermore, endocrine organs are susceptible targets for the action of genotoxic carcinogens.

The IARC Working Group of June 1991 (*2*) recognized that cell proliferation is an important mechanistic aspect for both genotoxic and nongenotoxic carcinogens. Cell proliferation may act at each stage of the carcinogenesis process, altering the size of the pool of cells at risk for a next event. Cell proliferation was considered to be a potentially important factor, espe-

cially as a part of the process of converting DNA adducts to mutation, as a potential enhancing factor of the mutation frequency by increasing the number of DNA errors during replication, and for determining dose-response relationships for some carcinogens. It was also recognized that not all agents that induce cell proliferation are necessarily involved in carcinogenesis; for example, *a*) not all skin hyperplasia-inducing compounds are skin tumor promoters, *b*) agents that induce regenerative cell proliferation appear to have different effects on tumor induction from agents that have a direct mitogenic effect, and *c*) the carcinogenic activity of many nonmutagenic agents depends on the continuous administration of the agent.

Although it has been proposed that enhanced cell replication is an enhancing factor in carcinogenesis either by favoring the expansion of altered cell clones or by increasing the rate of spontaneous errors in replication (*23–25*), it is necessary to acquire clearer and possibly quantifiable knowledge of carcinogenic mechanisms before cell proliferation per se becomes incorporated into an overall evaluation of carcinogenicity. There are numerous critical issues that have to be resolved before we can safely use an assessment approach with data on chemically induced cell proliferation. We should aim at understanding and quantifying the actual effects of enhanced cell proliferation and/or of reduced cell death rate on the number of spontaneous mutations. We should also try to clarify whether there are qualitative differences between DNA damage and repair that occur spontaneously and those induced by subliminal doses of carcinogens and whether and to what extent agents that enhance cell replication also control transformation. A clarification of the role of cell proliferation in the carcinogenesis process will help relieve the contradiction with which we are too often confronted; that is, although we agree that cancer has a multifactorial origin, we accept that risk assessments be geared to the effect of a single agent.

It is therefore essential that the testing of agents for carcinogenicity be elaborated and conducted with the advice and participation of basic scientists, as well as biostatisticians and epidemiologists. Discussing and criticizing the results of carcinogenicity studies *a posteriori* and multiplying their elaborated analysis will not substantially improve the quality of the data and will only marginally improve their interpretation.

REFERENCES

1. IARC. Approaches to Classifying Chemical Carcinogens According to Mechanism of Action. Internal Technical Report No. 83/001. Joint IARC/IPCS/CEC Report of a Working Group. International Agency for Research on Cancer, Lyon, 1983.
2. IARC. Mechanisms of Carcinogenesis in Risk Identification. Internal Technical Report No. 91/002. A Consensus Report of an IARC Monographs Working Group. International Agency for Research on Cancer, Lyon, 1991.
3. Vainio, H., Magee, P., McGregor, D., and McMichael, A. J., Eds. Mechanisms of Carcinogenesis in Risk Evaluation. IARC Scientific Publications No. 116, International Agency for Research on Cancer, Lyon, 1992.
4. Tomatis, L. Overview of perinatal and multigeneration carcinogenesis. In: Perinatal and Multigeneration Carcinogenesis (N. P. Napalkov, J. M. Rice, L. Tomatis, and H. Yamasaki, Eds.), IARC Scientific Publications No. 96, International Agency for Research on Cancer, Lyon, 1989.
5. Tomatis, L., Narod, S., and Yamasaki, H. Transgeneration transmission of carcinogenic risk. Carcinogenesis 13(2): 145–151 (1992).
6. Ackerknecht, E. H. Rudolph Virchow. The University of Wisconsin Press, Madison, WI, 1953.
7 Cohnheim, J. Lectures on General Pathology. The New Sydenham Society, London, 1889, pp. 746–821.
8. Friedewald, W. F., and Rous, P. The initiating and promoting elements in tumor production. J. Exp. Med. 80: 101–126 (1944).
9. Mottram, J. A developing factor in experimental blastogenesis. J. Pathol. Pact. 56: 181–187 (1944).
10. Berenblum, I. The mechanism of carcinogenesis: a study of the significance of co-carcinogenic action and related phenomena. Cancer Res. 1: 807–814 (1941).
11. Berenblum, I. A speculative review: the probable nature of promoting action and its significance in the understanding of the mechanism of carcinogenesis. Cancer Res. 14(7): 471–477 (1954).
12. Berenblum, I., and Shubik, P. A new quantitative approach to the study of the stages of chemical carcinogenesis in the mouse's skin. Br. J. Cancer 1: 383–391 (1947).
13. Boutwell, R. R. Some biological aspects of skin carcinogenesis. Prog. Exp. Tumor Res. 4: 207–250 (1964).
14. Foulds, L. Neoplastic development. Academic Press, London, 1969.
15. Slaga, T. J., Sivak, A., and Boutwell, R. K. Mechanisms of tumor promotion and co-carcinogenesis. Raven Press, New York, 1978.
16. Tannenbaum, A. The genesis and growth of tumors. Effects of caloric restriction per se. Cancer Res. 2: 460–467 (1942).
17. Strauss, B. S. The origin of point mutations in human tumor cells. Cancer Res. 52: 249–253 (1992).
18. Swenberg, J. A., Short, B., Borhoff, S., Strasser, J., and Charbonneau, M. The comparative pathobiology of alpha 2μ-globulin nephropathy. Toxicol. Appl. Pharmacol. 97: 35–46 (1989)
19. Dominick, M. A., Robertson, D. G., Bleavins, M. R., Sigler, R. E., Bobrowski, W. F., and Gough, A. W. Alpha 2μ-globulin nephropathy without nephrocarcinogenesis in male Wistar rats administered 1-(aminomethyl) cycloexaneacetic acid. Toxicol. Appl. Pharmacol. 111: 375–387 (1991).
20. Tennant, R. W., Elwell, M. R., Spalding, J. W., and Griesemer, R. A. Evidence that toxic injury is not always associated with induction of chemical carcinogenesis. Mol. Carcinog. 4: 420–440 (1991).
21. Huff, J. Absence of morphologic correlation between chemical toxicity and chemical carcinogenesis. Environ. Health Perspect. 101 (Suppl. 5): 45–54 (1993).
22. Huff, J. Chemical toxicity and chemical carcinogenesis. In: Mechanisms of Carcinogenesis in Risk Evaluation (H. Vainio, P. N. Magee, D. McGregor, and A. J. McMichael, Eds.), IARC Scientific Publications No. 116, International Agency for Research on Cancer, Lyon, 1992, pp. 437–475.
23. Cohen, S. M., and Ellwein, L. B. Genetic errors, cell proliferation and carcinogenesis. Cancer Res. 51: 6493–6505 (1991).
24. Loeb, L. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res. 51: 3075–3079 (1991).
25. Weinstein, B. Mitogenesis is only one factor in carcinogenesis. Science 251: 387–388 (1991).